C2

8 (Amended). The method according to claim 7, further comprising the step of purifying the isolated glycosylated human TNF-α.

10 (Amended). A composition consisting essentially of a purified glycosylated human TNF-α produced by the process of claim 3, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

C3

11 (Amended). In the method for treating a human disease or condition treatable by the administration of an effective amount of human TNF-α alone or in combination with other active principles or inactive carriers, diluents or excipients, the improvement wherein said human TNF-α is a purified glycosylated human TNF-α produced by the process of claim 3.

12 (Amended). Substantially homogeneous glycosylated TNF-α having cytotoxic biological activity, produced by the process of claim 2.

REMARKS

Claims 1-12 presently appear in this case. No claims have been allowed. The official action of April 15, 2002, has now been carefully considered. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to substantially homogeneous glycosylated human TNF-α having cytotoxic biological activity, as well as methods for the preparation of such a product by recombinant techniques, compositions and methods of use.

Claim 7 has been rejected under 35 U.S.C. §112, first paragraph, in reciting the limitation "variant thereof".

Claim 7 has now been amended to change "variant" to "mutant" to make the language correspond to the language of the remaining claims for which this rejection has previously been withdrawn. Accordingly, this rejection has now been obviated.

Claim 2 has been rejected as being vague and indefinite for reciting the term "capable".

Claim 2 has now been amended so as to delete use of this term, thus obviating this rejection.

Claim 7 has been rejected as being vague and indefinite for reciting the term "variant".

Claim 7 has now been amended to change the word "variant" to "mutant" to which the examiner has not objected. Accordingly, this rejection has now been obviated.

Claims 1, 2, 4, 7 and 12 have been rejected under 35 U.S.C. §102(b) as being anticipated by Korn. The examiner states that Korn demonstrated the cytotoxic activity of TNF produced by the cells by removing the supernatant from the transfected CHO clones and applying it to human fibroblast SV80 cells. This rejection is respectfully traversed.

The supernatant of Korn is not "isolated glycosylated human tumor necrosis factor-alpha". However, to make this point even more clear, the term "isolated" has been changed to "substantially homogeneous". The examiner's attention is invited to the definition of "substantially homogeneous" in the first paragraph on page 7 of the specification, which states:

> "Substantially homogeneous" glycosylated TNF means glycosylated TNF which is substantially free of other proteins native to the source from which the glycosylated TNF was isolated. This means that homogeneous glycosylated TNF is substantially free of ... other proteins of the cell or organism which serve as the synthetic origin of the glycosylated TNF, including whole cells and particulate cell debris.

The entire supernatant of Korn inevitably contains proteins from the CHO cells. Without isolating the TNF-α from the supernatant, one does not have "substantially homogeneous" glycosylated TNF-α. Accordingly, these claims are not anticipated by Korn. Reconsideration and withdrawal of this rejection are respectfully urged.

As to the examiner's reference to Fransen and Jue as describing biologically active TNF which is glycosylated, it should be noted that these references relate to murine TNF. This is irrelevant to the issue of whether or not TNF produced by CHO cells is glycosylated, as will be discussed further, below. The present specification establishes that human TNF produced by CHO cells is glycosylated. In any event, none of the references of record disclose substantially homogeneous TNF-α obtained from transfected CHO cells and, thus, the present claims cannot be anticipated by any of the references of record. Reconsideration and withdrawal of this rejection are, therefore, respectfully urged.

Claims 1, 2, 4 and 7-12 have been rejected under 35 U.S.C. §102(b) as being anticipated by Nakagawa. The examiner states that Nakagawa teaches a method for producing biologically active human TNF-β in CHO cells and teaches that this protein is glycosylated.

The present claims have now been amended to be directed solely to TNF-α. Thus, none of these claims can be anticipated by Nakagawa, which is directed to TNF-β. Reconsideration and withdrawal of this rejection are, therefore, respectfully urged.

Claims 3, 5, 6 and 8-11 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Korn in view of Allet. The examiner states that it would be expected that TNF produced by CHO cells is glycosylated and active. The examiner states that the statement of Allet that mature human TNF is not glycosylated is mere speculation. The examiner states that one of ordinary skill would have been motivated with a reasonable expectation of success to modify the methods of Korn because Allet teaches that recombinant TNF can be purified and formulated into compositions for treatment of human diseases. This rejection is respectfully traversed.

The examiner's case for obviousness is based on the assumption that it would be expected that TNF produced in CHO cells is glycosylated. However, many human proteins are non-glycosylated. If the human protein is non-glycosylated, it would not be expected that the protein produced in CHO cells would be glycosylated. Indeed, for naturally non-glycosylated proteins it is much more cost effective to produce it recombinantly in bacteria.

At the time of the present invention, those of ordinary skill in the art thought that human TNF-α was non-glycosylated. Regardless of the reason that the examiner cited the Allet et al patent, its disclosure is available for all purposes. At column 26, lines 14-17, Allet states:

> Mature human TNF is believed to be non-glycosylated. This belief is supported by the absence of a glycosylation signal in our human TNF coding sequence.

See also U.S. Patent 6,300,358 to Cioli, copy attached, which states at column 1, lines 27-33:

> TNF is a non-glycosylated polypeptide having relative molecular mass (M_r) of 17,500 (17.5 KDa) and known amino acid sequence ...

Also attached is the cover page and columns 1 and 2 of U.S. Patent 5,846,763 to Lee et al. Note column 1, lines 50-52, where it states:

> Natural human TNF is a 157 amino acid, non-glycosylated protein with a molecular weight of approximately 17 kDa under denaturing conditions.

The examiner cites Fransen and Jue as disclosing murine TNF as being glycosylated. However, attached hereto is Marmenout et al, "Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor", Eur J Biochem 152(3):515-522 (1985). Note the abstract of Marmenout states:

> In contrast to mouse TNF, [human TNF] contains no potential N-glycosylation site.

See also page 520, in the paragraph briding the columns, where Marmenout says:

> The molecular mass of the mature TNF polypeptide as predicted from the cDNA sequence (17356 Da) is in close agreement with a value of 17 kDa obtained by SDS/polyacrylamide gel electrophoresis of natural, human TNF. These data, together with the absence of an *N*-glycosylation signal (in contrast to mouse TNF [22], see also Fig. 5) suggest that human TNF is not glycosylated. [endnote omitted]

Thus, the mouse sequence has N-glycosylation sites and would be expected to be glycosylated. However, as the human TNF has no

potential N-glycosylation sites, it is not expected to be glycosylated, as stated by Allet. This is not mere speculation but is based on the data discussed in Marmenout. In contrast to this evidence supplied by applicants, the examiner has submitted no evidence suggesting that human TNF-α is glycosylated.

The present invention is the first time that it has been discovered that human TNF-α is, indeed, glycosylated, thus, for the first time, providing motivation to actually produce and isolate substantially homogeneous TNF-α in CHO cells. If the art at the time that the present invention was made expected that human TNF-α was not glycosylated, then there would have been no motivation to produce and isolate it from CHO cells as the same protein could have been produced and isolated much more inexpensively in *E. coli*, which bacterial recombinant production was already well known at the time of the present invention.

The fact that Allet discloses procedures that could be used to purify substantially homogeneous TNF-α from the supernatant of Korn does not provide motivation to do so. As the CHO which would be obtained by purifying the supernatant of Korn would not be expected to have properties any different from that already available from prior art sources, there would be no motivation to purify the supernatant of Korn. Without such motivation, the process and product of the present invention would not have been obvious in the sense of 35 U.S.C. §103. See MPEP §2143.01. Reconsideration and withdrawal of this rejection are, therefore, respectfully urged.

Claims 3, 5, 6 and 8-11 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Nakagawa in view of Allet. This rejection is respectfully traversed.

In view of the fact that Nakagawa relates only to TNF-β and the claims have now been amended to be directed only to TNF-α, this rejection has now been obviated. No combination of Nakagawa with Allet would teach the obviousness of the production of glycosylated TNF-α.

Accordingly, it is submitted that all the claims now present in the case clearly define over the references of record and fully comply with 35 U.S.C. §112. Reconsideration and allowance are, therefore, earnestly solicited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By
Roger L. Browdy
Registration No. 25,618

RLB:rd
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
G:\BN\I\intp\Messikal\Pto\AmendmentD.doc

Version with Markings to Show Changes Made

Claims 1-3, 5-8 and 10-12 have been amended as follows:

1 (~~Amended~~Twice-amended). ~~Isolated~~ Substantially homogeneous glycosylated human tumor necrosis factor-alpha (TNF-α) having cytotoxic biological activity.

2 (~~Twice~~Thrice-amended). A method for preparing ~~isolated~~ substantially homogeneous glycosylated human tumor necrosis factor-alpha (TNF-α) exhibiting cytotoxic biological activity, comprising:

(a) ligating DNA encoding human ~~TNF~~TNF-α, or a mutant thereof exhibiting cytotoxic biological activity, which mutant ~~is capable of having~~has its cytotoxic biological activity neutralized by antisera raised against human glycosylated ~~TNF~~TNF-α, to a replicable expression vehicle to obtain a replicable recombinant DNA comprising said DNA and said replicable expression vehicle;

(b) transforming eukaryotic cells with said replicable recombinant DNA to form transformants;

(c) culturing said transformants to cause said transformants to express said glycosylated human ~~tumor necrosis factor~~TNF-α; and

(d) isolating said glycosylated human ~~tumor necrosis factor~~TNF-α from the cultured transformants.

3 (Amended). The method according to claim 2, further comprising the step of purifying the isolated glycosylated human ~~tumor necrosis factor~~TNF-α.

5 (~~Twice~~Thrice-amended). A composition consisting essentially of glycosylated human tumor necrosis factor-alpha (TNF-α) having cytotoxic biological activity and at least one pharmaceutically acceptable carrier, diluent, or excipient.

6 (~~Amended~~Twice-amended). In the method for treating a human disease or condition treatable by the administration of an effective amount of human tumor necrosis factor-alpha ~~TNF~~ (TNF-α) alone or in combination with other active principles or inactive carriers, diluents or excipients, the improvement wherein said human ~~TNF~~ TNF-α is glycosylated human ~~TNF~~ TNF-α exhibiting cytotoxic biological activity.

7 (~~New~~Amended). A method in accordance with claim 2, wherein said DNA encoding human ~~TNF~~ TNF-α or a ~~variant~~ mutant thereof encodes human ~~TNF~~TNF-α.

8 (Amended~~New~~). The method according to claim 7, further comprising the step of purifying the isolated glycosylated human ~~tumor necrosis factor~~TNF-α.

10 (Amended~~New~~). A composition consisting essentially of a purified glycosylated human ~~tumor necrosis factor~~TNF-α produced by the process of claim 3, and at least

one pharmaceutically acceptable carrier, diluent, or excipient.

11 (AmendedNew). In the method for treating a human disease or condition treatable by the administration of an effective amount of human TNF TNF-α alone or in combination with other active principles or inactive carriers, diluents or excipients, the improvement wherein said human TNF TNF-α is a purified glycosylated human tumor necrosis factorTNF-α produced by the process of claim 3.

12 (AmendedNew). Isolated Substantially homogeneous glycosylated tumor necrosis factorTNF-α having cytotoxic biological activity, produced by the process of claim 2.